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Do early environmental experiences impact numerical discrimination abilities in a lizard?

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Corresponding Author:	Pablo Recio Australian National University College of Science: Australian National University Madrid, Madrid AUSTRALIA
Order of Authors:	Pablo Recio
	Dalton C. Leibold
	Ondi L. Crino
	Cristopher R. Friesen
	Amelia Y. Peardon
	Daniel W. A. Noble
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Australian National University College of Science: Australian National University
Corresponding Author's Secondary Institution:	
First Author:	Pablo Recio
First Author Secondary Information:	
Order of Authors Secondary Information:	
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Suggested Reviewers:	Catarina Vila Pouca catarinavilapouca@gmail.com

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	Expert in cognition and the effects of early conditions on numerical discrimination
	Enrique Font Bisier enrique.font@uv.es Expert in reptiles' cognition
	Isabel Damas-Moreira isabeldamas.m@gmail.com Expert in reptiles' cognition
	Maria Elena Miletto Petrazzini mariaelena.milettopetrazzini@gmail.com Expert in numerical cognition
	Martin Whiting martin.whiting@mq.edu.au Expert in reptiles' cognition
	Pau Carazo pau.carazo@uv.es Expert in reptiles' cognition
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Do early environmental experiences impact numerical discrimination abilities in a lizard?

Pablo Recio[1],‡ , Dalton C. Leibold[1], Ondi L. Crino [1,2], Christopher R. Friesen[3], Amelia Y. Peardon [1], Daniel W.A. Noble[1]

[1] Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

[2] Flinder's University, College of Science and Engineering, Bedford Park, SA 5042, Australia

[3] University of Wollongong, Wollongong, NSW 2500, Australia

‡ Corresponding author: pablo.reciosantiago@anu.edu.au

ORCID:

Pablo Recio ORCID: 0000-0002-5890-0218

Dalton C. Leibold ORCID: 0000-0001-9645-2033

Ondi L. Crino ORCID: 0000-0001-5700-1387

Christopher R. Friesen ORCID: 0000-0001-5338-7454

Amelia Y. Peardon ORCID: 0009-0005-6227-8379

Daniel W.A. Noble ORCID: 0000-0001-9460-8743

Keywords

Numerical discrimination, Corticosterone, Temperature, Reptiles, Early life stress, Cognitive abilities

Abstract

From social behaviour to navigating complex environments, quantitative abilities can be crucial to fitness. However, early life conditions can impact brain development to affect quantitative competence. For example, early thermal conditions or glucocorticoid concentrations (GCs) – stress-related hormones transmitted by the parents – can play a prominent role in shaping cognition through their effects on brain physiology. Furthermore, temperature can lead to increased levels of GCs that can be transmitted to offspring, emphasizing the need to study the combined effects of these two factors. Here, we investigated the effects of elevated prenatal corticosterone (CORT) – the main GC in reptiles – and incubation temperature on numerical discrimination in the common garden skink (*Lampropholis guichenoti*). We assessed numerical abilities through a spontaneous choice test with food as a stimulus. Employing a repeated measures design, we subjected lizards to five numerical tests each differing in the ratios between the two choices (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4). Contrary to our predictions, we found no evidence for treatment effects on lizard behaviour and no use of numerical discrimination during foraging in *L. guichenoti*. Our findings build on previous studies suggesting that reptiles feeding on live prey may have difficulty distinguishing between different prey quantities. Despite our results, further investigation of numerical abilities in live-prey-feeding reptiles and the impact of early conditions on other taxa is warranted, as these areas remain understudied.

Significance statement

Discriminating between quantities can be crucial for individual fitness, yet the impact of early environmental factors on the development of quantitative competence remains largely unexplored. In this study, we investigated, for the first time, the effects of prenatal corticosterone exposure and incubation temperature on numerical discrimination in a reptile. Contrary to our predictions, we did not observe any evidence of numerical discrimination and therefore no impact of early developmental environment. Our results align with previous studies showing that reptiles consuming live prey struggle to differentiate prey numbers in spontaneous choice tests. These results open new avenues for exploring the evolution of numerical abilities in reptiles and identifying factors influencing the development of quantitative skills in this group. Further research should explore alternative methods, such as trained tasks, to better understand the cognitive mechanisms underlying quantitative competence in reptiles.

Introduction

The ability to discriminate between quantities is a fundamental cognitive skill widely observed across the animal kingdom (Vonk and Beran 2012; Stancher et al. 2015; Beran and Parrish 2016). Numerical cognition can contribute to social decisions, mate selection, and foraging behaviour, which can be adaptive in various ecological contexts (Lucon-Xiccato and Dadda 2017; Nieder 2018). For instance, female lions (*Panthera leo*) use numerical information to assess the risk of confronting rival groups (McComb et al. 1994), male adult mealworms (*Tenebrio molitor*) select sites with scents of more females (Carazo et al. 2009), and red-backed salamanders (*Plethodon cinereus*) prefer tubes containing larger numbers of prey (Uller et al. 2003). Given the adaptive advantages of numerical and quantity discrimination, research has

61 focused extensively on exploring the ability of different species to differentiate between
62 quantities employing different contexts and paradigms.

63 Numerical competence is often evaluated by considering the types of cues animals use (Plotnik
64 et al. 2019; Cooper et al. 2024), the ecological contexts in which these abilities are deployed
65 (McComb et al. 1994; Chittka and Geiger 1995; Uller et al. 2003; Carazo et al. 2009), and the
66 neural mechanisms that underlie them (Nieder 2005). The latter have demonstrated that non-
67 verbal numerical representations in mammals activates an extensive neural pathway including
68 regions involved in eye movement, attention control, or complex learning (Nieder 2005). The
69 connection between numerical discrimination and brain physiology underscores the importance
70 of understanding how brain development influences numerical abilities.

71 Brains are particularly sensitive to environmental inputs during early stages of development
72 (Coomber et al. 1997; Zhu et al. 2004), with long-lasting effects on cognitive abilities (Zhu et al.
73 2004; Amiel et al. 2014). For instance, glucocorticoids (GCs), steroid hormones that mediate the
74 physiological stress response (Sapolsky et al. 2000), can alter performance in spatial, associative,
75 or reversal learning tasks if elevated during early stages of development (Szuran et al. 1994; Zhu
76 et al. 2004; Crino et al. 2014; Farrell et al. 2015; Bebus et al. 2016; Lui et al. 2017). In
77 ectotherms, temperature can affect GC levels in parents, cascading into effects on the developing
78 offspring [i.e., maternal effects; Jessop et al. (2016); Racic et al. (2020); Mentesana and Hau
79 (2022); Crino et al. (2023)]. Prenatal exposure to GCs is typically associated with impaired
80 learning skills, but these effects have been shown to vary depending on factors such as dose, sex,
81 or type of cognitive test employed (Szuran et al. 1994; Farrell et al. 2015; Bebus et al. 2016; Haq
82 et al. 2021). Most past studies have focused on the effects of GC exposure during development
83 on spatial, associative, or reversal learning. Few studies have focused on how exposure to GCs

84 during development can affect quantitative abilities, despite the importance of numerical
85 discrimination.

86 Although maternally transmitted GCs are a significant factor in cognitive development, other
87 environmental conditions can also influence cognition, either independently or through
88 interactions with GCs. For instance, the early thermal environment plays a prominent role in
89 brain development and cognitive abilities in ectotherms (Coomber et al. 1997; Amiel and Shine
90 2012; Clark et al. 2014; Amiel et al. 2014; Amiel et al. 2017; Vila Pouca et al. 2018, 2019). In
91 lizards, incubating eggs at elevated temperatures improves cognitive abilities consistent with
92 elevated neuronal density in some areas of the brain (Amiel and Shine 2012; Clark et al. 2014;
93 Amiel et al. 2014; Amiel et al. 2017), but impairs cognition at extreme temperatures (Dayananda
94 and Webb 2017; Abayarathna and Webb 2020). In addition, when Vila Pouca et al. (2019) tested
95 the effects of elevated incubation temperature on numerical discrimination in Port-Jackson
96 sharks (*Heterodontus portusjacksoni*) and found that sharks incubated at elevated temperatures
97 learned faster in a quantity discrimination task (Vila Pouca et al. 2019). However, the effects of
98 early thermal environment on numerical abilities in other taxa remains largely unexplored.

99 Early-life GCs and thermal conditions could have interactive effects that influence cognitive
100 development. Understanding how these factors influence quantitative abilities could reveal
101 important insights into the ecological relevance of numerosity. Given the influence of numerical
102 cognition on fitness, we would expect evolutionary pressures to be similar across taxa, and yet,
103 research on numerical discrimination has been traditionally focused on mammals, birds, and
104 fishes (Nieder 2005; Agrillo and Bisazza 2014). However, recent studies demonstrate that
105 reptiles are also competent in numbers. For example, red-footed turtles (*Chelonoidis carbonaria*)
106 rapidly learned to associate colour with higher volumes of food (Soldati et al. 2017), Hermann's

tortoises (*Testudo hermanni*) discriminate between large and small food quantities in a spontaneous choice test (Gazzola et al. 2018), and freshwater turtles (*Trachemys scripta* and *Mauremys sinensis*) rapidly learned a quantity discrimination task (Lin et al. 2021, 2024). The Italian wall lizard (*Podarcis siculus*) failed to discriminate between different numbers of food items in a spontaneous choice test (Miletto Petrazzini et al. 2017), but succeeded in a trained quantity discrimination task (Miletto Petrazzini et al. 2018). In contrast, Iberian rock lizards (*Iberolacerta cyreni*) preferred larger quantities of crickets when presented five versus ten items (Recio et al. 2021). Indeed, the literature shows evidence for basic quantitative abilities in reptiles that may vary with the type of cue and the test employed.

Here, we investigated the effect of prenatal corticosterone (CORT) - the main GC in reptiles - and incubation temperature on numerical discrimination in a lizard. We manipulated CORT concentration (CORT-treated or a sham control) in *L. guichenoti* eggs and incubated them under one of two temperature regimes (Cold - 23 ± 3ºC or Hot - 28 ± 3ºC) in a 2x2 factorial design. Then, we assessed numerical discrimination abilities of lizards using a spontaneous choice test approach. The numerical discrimination task consisted of five tests that differed in the relative number of food items: 1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 crickets. We based this experiment on observations in nature that the ability to detect a change in stimulus intensity depends on the relative, not absolute, difference between the stimuli ('Weber's law'; Agrillo and Bisazza (2014)). Built upon other studies that explored the cognitive effects of prenatal GCs or thermal early environment in reptiles and other taxa, we hypothesized that lizards incubated at higher temperatures would outperform those incubated at lower temperatures in the numerical discrimination task (Amiel and Shine 2012; Clark et al. 2014; Amiel et al. 2014; Amiel et al. 2017; Vila Pouca et al. 2019). We predicted that increased CORT levels would impair numerical

competence (Szuran et al. 1994; Zhu et al. 2004), with more pronounced effects at lower temperatures. Consistent with Weber's law, we predicted that lizards incubated at higher temperatures would successfully discriminate between quantities even at high ratios (e.g. 3 VS 4), whereas even the lowest ratios (e.g. 1 VS 4) could be challenging for those exposed to CORT or incubated at lower temperatures.

Methods

Animal husbandry

Breeding colony – The lizards tested in our experiment came from a breeding colony established in the lab in 2019. The colony consisted of approximately 180 adults of *L. guichenoti* housed in communal containers (41.5 L x 30.5 W x 21 H cm) with six lizards (2 males and 4 females) per enclosure. Enclosures were provided with non-stick matting, shelter, and several small water dishes filled daily, and lizards were fed mid-size crickets (*Acheta domestica*) three days a week. Crickets were dusted with calcium weekly and multivitamins and calcium biweekly. Room temperatures were set to 22-24ºC, but to ensure a temperature gradient, we employed a heat chord and a heat lamp following a 12 h light:12 h dark cycle, keeping the warm side of enclosures at 32-34ºC.

Egg collection and incubation – Between mid-October 2023 to the end of February 2024, we placed small boxes (12.5 L x 8.3 W x 5 H cm) containing vermiculite on one side of the communal enclosures (see above) to provide females with a place to lay the eggs. We checked for eggs in the boxes three days a week. Egg length and width were measured with a digital calliper to the nearest mm (± 0.1 mm), while mass was recorded with a digital scale (OHAUS, Model spx123) to the nearest g (± 0.001 g). Eggs were assigned a clutch number and an

individual identity. Then we treated eggs with CORT or a vehicle control (see *Manipulating Early Thermal and CORT Environments* details below) and placed them in individual cups (80 mL) with moist vermiculite (12 g water to 4 g vermiculite). We covered the cups with plastic wrap to retain moisture and left the eggs in incubators (LATWIT 2X5D-R1160) programmed to two different thermal regimes (see *Manipulating Early Thermal and CORT Environments* details below) until hatching. Incubators were checked for the presence of hatchlings three times a week.

Hatchlings – Immediately after hatching, we measured Snout-Vent Length (SVL) and Tail Length (TL) with a ruler to the nearest mm (± 0.1 mm), and mass using a digital scale (OHAUS, Model spx123) to the nearest g (± 0.001 g). Hatchlings were then placed in individual enclosures (18.7L x 13.2W x 6.3H cm) provided with non-stick matting and a small water dish. During this period, they were given water daily and received 3-6 small *A. domestica* crickets three times a week. All care otherwise follows similar protocols to adults (see above).

Manipulating Early Thermal and CORT Environments

We manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (Cold - 23 ± 3 ºC or Hot - 28 ± 3 ºC) in a 2x2 factorial design (Fig. 1 A). Eggs were allocated to one of two different developmental stress treatments: CORT-treated eggs were topically supplied with 5 µL of a CORT solution (10 pg/mL). Control treatment eggs received an equal volume of 100% Ethanol. Corticosterone treatments were made by dissolving crystalline corticosterone (Sigma, Cat. No. C2505) in 100% ethanol. We selected doses based on previous studies where CORT treatment increased mean yolk CORT levels ~3.7x higher than control eggs in *Lampropholis delicata* (Crino et al. 2024). Then, eggs were incubated in one of the two

174 previously mentioned temperature regimes (Cold or Hot) until hatching. These temperatures
175 represent the upper and lower limit of the natural incubation temperatures for this species (Qualls
176 and Shine 2000).

177 The number of eggs per clutch assigned to each hormone and temperature treatment was
178 counterbalanced in a partial split-clutch design. At least one egg per clutch to each treatment, and
179 the remaining eggs were randomly assigned to one of the treatments. When we found less than
180 four eggs in a clutch, we assigned each egg randomly to one of the treatments.

181 *Numerical discrimination task*

182 Two weeks before starting the numerical discrimination task (see below), lizards were moved to
183 the experimental arenas for acclimatisation. The arenas were individual medium-size (41 L x
184 29.7 W x 22 H cm) plastic containers provided with a shelter (9 L x 6 W x 1.5 H cm) on one of
185 the sides and a water dish in the center (Fig. 1 B). Arenas were placed on seven different racks
186 located in two experimental rooms. Each rack was associated with its own CCTV system (device
187 model DVR-HP210475), allowing us to record lizards' behaviour during the experiment. Despite
188 the conditions in the experimental rooms were the same as in the colony room, we
189 counterbalanced the number of lizards per treatment in each rack to control for any potential
190 effects of the room or the position of the lizard in the rack. During acclimatisation and
191 throughout the experiment, lizards were fed with only one cricket daily, dusted with calcium and
192 multivitamins (see protocol below), and water was supplied *ad libitum*. The temperature of both
193 rooms was set to 22-24ºC, but we also provided a temperature gradient (24-32°C) using a heat
194 cord along the center of the arena and heat lamps in a 12 h light: 12 h dark cycle.

The numerical discrimination test consisted of a series of two-choice trials where lizards were presented with different numbers of food items. Each choice was placed on one of the two lateral platforms of a white 3D printed device (see Fig. 1 B, C) and then covered with a transparent Petri dish to prevent the lizard from obtaining the food. We performed five tests that differed in the ratio between both choices of crickets. Specifically, we compared: 1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4 crickets. The food consisted of small frozen crickets (*A. domestica*) that were thawed and dusted with calcium and multivitamins. We decided to use frozen crickets to avoid movement that would affect lizard behaviour (Cooper et al. 2024).

Lizards were fed frozen crickets for one month prior to the experiments to habituate them to eating frozen prey. To habituate lizards to the experimental conditions, frozen crickets were placed on top of one of the platforms and Petri dishes during each feeding for two weeks prior to experimental trials. The side where the food was placed was changed every day to avoid any side bias during the trials. However, to further control for side biases, the higher number of crickets was randomly placed on the right or the left platform. We also counterbalanced the number of individuals per treatment tested in the same type of trial each day.

The crickets used during the experiment were all the same size, but to control for other quantitative traits we changed the orientation of the crickets as illustrated in Fig. 1 D. This approach created a conflict of information between choices, as horizontally oriented crickets could occupy the same space as a greater number of vertically oriented crickets. This allowed us to determine whether the lizards relied on discrete numerical units rather than non-numerical cues, such as total length, area, or volume (Agrillo and Bisazza 2014). In addition, after the experiments, we conducted another set of trials to test whether the orientation of the crickets

could affect lizards' choice, but we found no effect of orientation (see *Supplementary Material - Prey orientation test*).

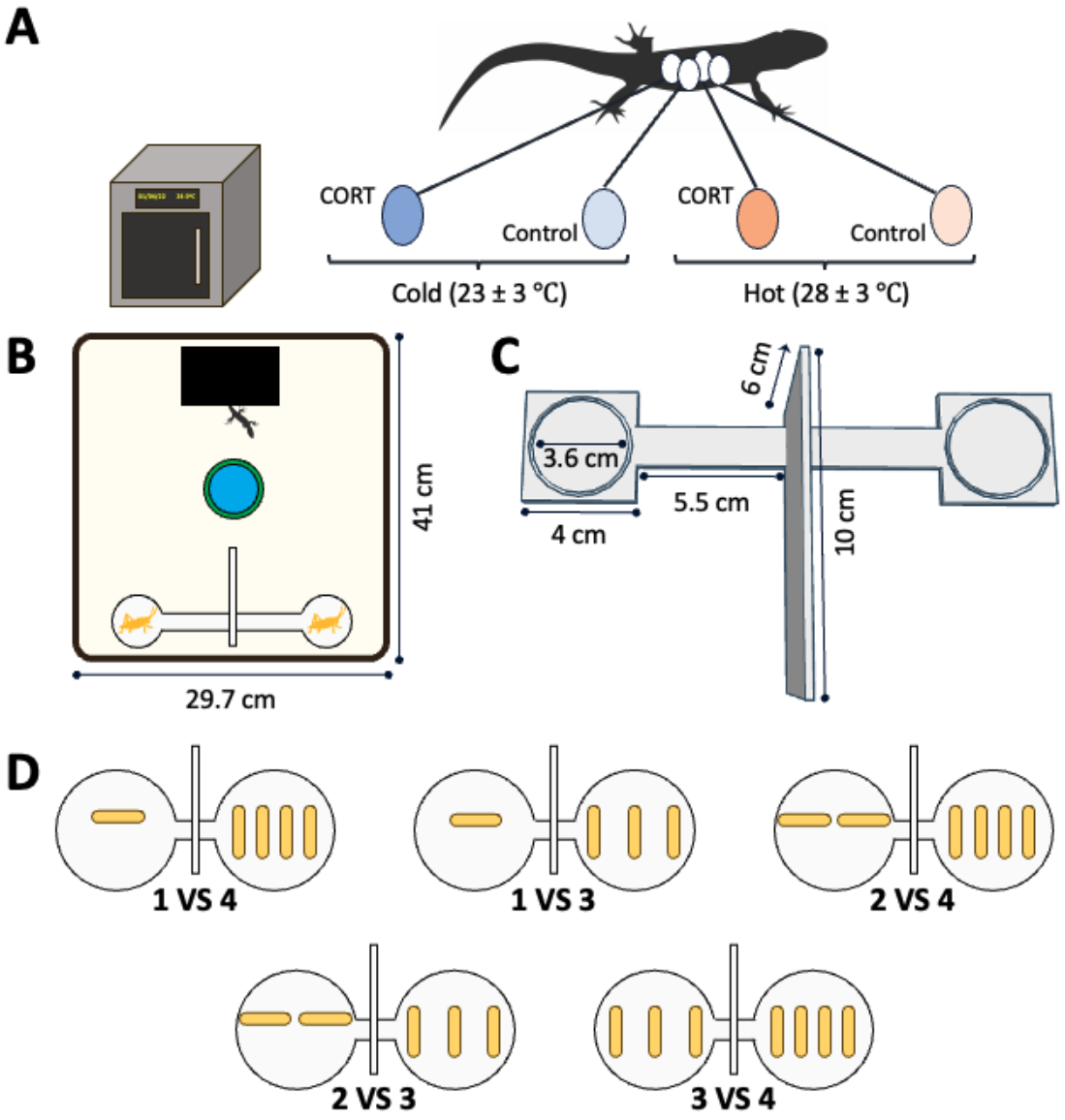

Fig 1— Experimental design. Panel (**A**) shows the early environment manipulation procedures. (**B**) illustrates the arena where the tests were performed, while panel (**C**) indicates the measurements of the platform used for the experiments. Finally, panel (**D**) displays the types of numerical tests used and the orientation of the crickets in each test.

All tests were done between 1000-1200 hours when the lizards were most active. Each trial was performed daily for five days between the 17th and 22nd of May 2024. We recorded three main variables: i) *Latency*, defined as the time between the start of the test - when the platform was left in the enclosure - until the first interaction with one of the options; ii) First choice of the lizard (*Choice*), recorded as 1 if the lizard interacted first with the larger amount or 0 if not; and iii) *Interest* shown for the higher amount of food (detailed below). We considered that the lizard interacted with the food when it touched the Petri dish with its snout or when the front half of the body was on top of the platform. For five minutes after the first interaction with one of the options, we recorded the time spent interacting with that option for a maximum of five minutes; we did this for both amounts of food independently of what was the first choice. We measured the interest shown for the higher amount of food (*Interest*) by subtracting the time interacting with the option with fewer crickets to the time spent interacting with the larger amount of food. If there was no interaction with one of the options during the test, we considered the time spent as zero for that option. When there was no interaction with any of the choices, both *Latency*, *Choice*, and *Interest* were noted as NA. We also considered Interest as NA in a few cases where the animals managed to lift the dish and eat the prey; Latency and Choice were recorded and employed in the analyses. We excluded from the analyses all the individuals that did not make any choice in three or more tests. All the analyses were performed by PR, who was blind to the treatment of the lizards.

Motivation plays a key role in animals' performance in spontaneous choice tests (Agrillo and Bisazza 2014; Bisazza et al. 2014). To ensure that lizards remained motivated to forage, they were provided with only one cricket during both acclimatization and the numerical task (see above). During acclimatization, lizards were fed at the same time each day as the experiments

were conducted. In the numerical task, lizards received a cricket immediately after the test and then fasted for more than 20 hours, which we expected would help standardize fasting levels across individuals. To further control for motivation, we excluded from our analyses any trials in which lizards did not interact with any options or did not eat the cricket provided after the test (n = 0 in both cases). Consequently, we do not consider motivation to be a major factor influencing our results (but see Discussion).

Statistical analyses

In our analyses, we fitted a multivariate Bayesian multilevel model using the brm function from the *brms* package (Bürkner 2017) in R (version 2.8.2) (R Core Team 2021). We ran four parallel MCMC chains of 3000 iterations for each model, with a warmup period of 1000 iterations. We checked that all MCMC chains converged (Rhat < 1.2) and were mixing effectively to ensure we had >1000 effective samples from the posterior distribution.

We modelled the three main recorded behaviours: *Latency*, *Choice*, and *Interest* as the response variables. We assumed normal distributions for Latency (log-transformed) and Interest [family = gaussian()]. The error structure for Choice was modeled using a Bernoulli distribution with a logit link function [family = Bernoulli(link = 'logit')]. We included test (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4), hormone (CORT versus Control), incubation temperature (Cold versus Hot), and the three-way interaction as fixed factors. We also included in our predictors sex, and age. We did not find any effect of sex or age on Choice or Interest, but we found an effect on Latency (see Results). Regardless, we included both factors in all models. Age was centered to zero in the model, and we averaged the predicted values for males and females.

We included lizard identity and clutch as random factors. *L. guichenoti* lays up to two clutches per year (Joss and Minard 1985). Since egg collection was done during half of the breeding season, each clutch likely came from a unique mother, and so, clutch identity captures potential maternal effects.

We used the posterior distributions of parameters from these models to test for between treatment differences in the ability to discriminate different numbers of crickets for each of the numerical tests. Because the posterior distributions were not always normally distributed, we report the median as the main measure of central tendency. We used the 95% Highest Posterior Density Intervals (95% HPDI) using the hdi function in bayestestR (Makowski et al. 2019) to test if the contrasts between treatments for all the variables, or the *Interest* per each test was different form zero. Given Choice was Bernoulli, we modified our null hypotheses to test if differed from a probability of 0.5 as there were only two possible choices and if animals were choosing randomly we would predict each choice would have a 50% probability.

Ethical note

Subjects were collected from a breeding colony that had been kept in the lab since 2019. All animals in this experiment were provided humane laboratory housing, thermoregulation opportunities, and light and humidity conditions (see above for details). Lizards in the colony received five to seven crickets per individual three times a week while the subjects in the experiment were fed once per day. All lizards were handled minimally to avoid stress. Experimental procedures and laboratory housing complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee

(A2022_33). Upon completion of experimental trials, lizards were kept in their enclosure and will be euthanized following standard humane procedures once they reach the humane endpoint.

Results

Overall, males showed lower latencies than females (median of the estimated effect of sex = -0.45, 95% HPDI = [-0.89, -0.02]), and older individuals also made decisions faster (mean estimated effect of age = 0.06, 95% HPDI = [0.01, 0.11]; see Table S3 in *Supplementary Material*). However, we did not see any effect of age or sex on *Choice* or *Interest* (see *Supplementary Material*).

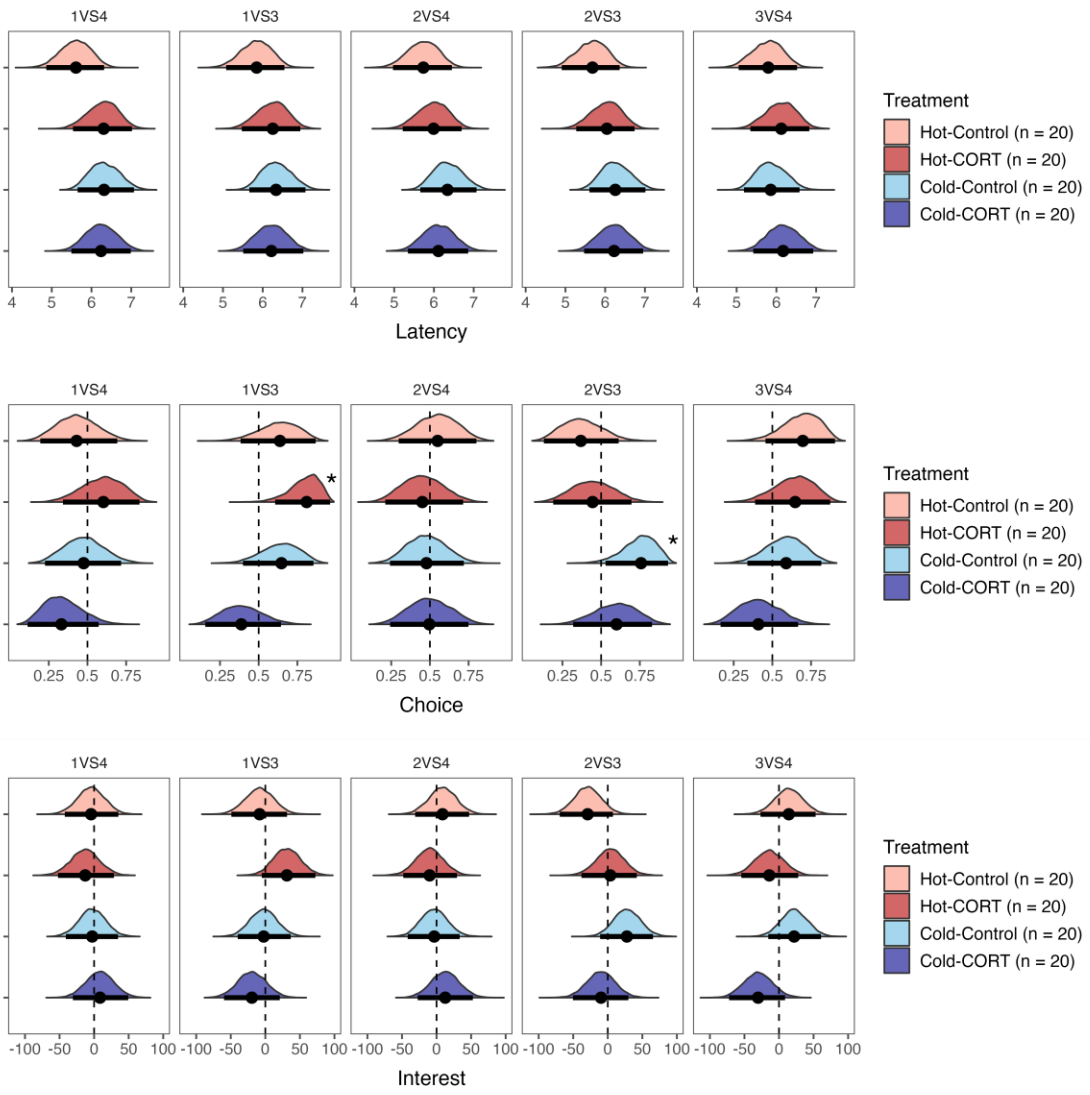

Fig 2— Estimates of log-latency (Latency), the probability of choosing the larger number of crickets first (Choice), and the estimated interest in the larger number of crickets (Interest) for each of the numerical tests performed across different developmental treatments. The x-axis represents the estimate, and the y-axis is the posterior density of the estimates. The different colours indicate the different treatments. Points and bars represent the median and 95% Higest Density Intervals (95% HPDI) of the estimates, respectively. Vertical dashed lines in Choice and Interest graphs values 0.5 and 0, respectively. Asterisks indicate values significantly different

from 0.

Neither temperature, CORT, or their interaction affected performance in any of the tests, nor were there any clear patterns suggestive of an effect of the developmental environment on numerical discrimination (see Table 1 and Fig. 2). Except for Hot-CORT lizards in 1 VS 3 tests or Cold-Control lizards in 2 VS 3 tests, the predicted probability of choosing the larger number of crickets first was not different from 0.5 (Fig. 2; but also see Table S1 and Table S2 in *Supplementary Material*). Similarly, interest in the larger number of crickets (*Interest*) was not different from 0 in any of the tests (Fig. 2; but also see Table S1 and Table S2 in *Supplementary Material*).

Table 1. Effects of temperature, CORT, and their interaction on Latency, Choice, and Interest in each of the numerical discrimination tests. The table shows the contrasts for each predictor (Temperature = [medianHot - medianCold]; Hormone = [medianControl - medianCORT]; and their Interaction = [(medianHot-Control - medianHot-CORT) - (medianCold-Control - medianCold-CORT)]). 95% Higest Density Intervals (95% HPDI) test the hypothesis that contrasts are different from zero.

		Tests				
Variable	Predictor	1VS4	1VS3	2VS4	2VS3	3VS4
Latency	Hormone	-0.3 [-1.39 , 0.8]	-0.1 [-1.14 , 0.82]	0 [-0.97 , 0.97]	-0.2 [-1.08 , 0.77]	-0.3 [-1.17 , 0.52]
log(latency)	Temperature	-0.3 [-1.45 , 0.74]	-0.2 [-1.23 , 0.73]	-0.4 [-1.36 , 0.57]	-0.4 [-1.33 , 0.52]	-0.1 [-0.94 , 0.77]

		Tests				
Variable	Predictor	1VS4	1VS3	2VS4	2VS3	3VS4
	Interaction	-0.8 [-1.94 , 0.4]	-0.5 [-1.75 , 0.64]	-0.5 [-1.7 , 0.74]	-0.4 [-1.57 , 0.79]	0 [-1.2 , 1.23]
Choice	Hormone	0 [-0.45 , 0.43]	0 [-0.43 , 0.53]	0 [-0.33 , 0.41]	0.1 [-0.36 , 0.45]	0.1 [-0.25 , 0.48]
log(odds)	Temperature	0.1 [-0.34 , 0.54]	0.2 [-0.29 , 0.66]	0 [-0.35 , 0.39]	-0.3 [-0.66 , 0.14]	0.2 [-0.2 , 0.53]
	Interaction	-0.3 [-0.79 , 0.21]	-0.4 [-0.87 , 0.07]	0.1 [-0.4 , 0.61]	-0.2 [-0.72 , 0.26]	-0.1 [-0.6 , 0.37]
Interest	Hormone	-1.3 [-59.55 , 54.62]	-11.9 [-83.67 , 64.05]	1.5 [-59.81 , 65.18]	2.5 [-78.71 , 82.35]	40.3 [-19.82 , 99.74]
	Temperature	-11.3 [-68.87 , 46.46]	22.6 [-50.44 , 95.41]	-5.1 [-68.08 , 56.31]	-22.4 [-102.22 , 59.51]	3.5 [-55.15 , 65.4]
	Interaction	19.8 [-56.91 , 94.76]	-56.5 [-133.25 , 20.49]	34.5 [-41.72 , 110.47]	-70.2 [-145.33 , 6.71]	-23.9 [-100.36 , 55.25]

Discussion

Contrary to our predictions that numerical cues would influence foraging decisions, and that elevated prenatal CORT or cold incubation temperatures would impair numerical discrimination, we found no significant differences across tests or treatments. Our results indicate that lizards did not discriminate between different numbers of crickets, nor did their performance vary under our

312 experimental conditions. Overall, these findings suggest that *L. guichenoti* did not use numerical
313 or other potential quantitative cues for foraging decisions.

314 *Foraging context may influence prey number discrimination in reptiles*

315 Lack of numerical discrimination in *L. guichenoti* may be due to insectivorous lizards relying on
316 other cues, such as movement or size, for foraging decisions. In studies where reptiles
317 discriminated between different amounts of food, the relevant stimuli involved either vegetables
318 (Gazzola et al. 2018; Szabo et al. 2021) or large quantities (e.g., 5 vs. 10)(Recio et al. 2021). In
319 contrast, when *Podarcis siculus* were presented different numbers of *Musca domestica* larvae in
320 a spontaneous choice test, the lizards failed to discriminate between quantities despite showing
321 high quantitative skills when prey size was the main cue (Miletto Petrazzini et al. 2017) or when
322 tested using training procedures (Miletto Petrazzini et al. 2018). Notably, the numbers of food
323 items employed in Miletto Petrazzini et al. (2017) were always fewer than four. Spontaneous
324 choice tests assume that animals should select the most appropriate quantity, which may not
325 necessarily be the largest one (Agrillo and Bisazza 2014). This occurrs if the costs of selecting
326 the larger option outweigh the benefits. For example, when presented two groups with different
327 numbers of conspecifics or if prey handling costs are related to larger quantitative traits like size
328 (Agrillo and Bisazza 2014). It could be that the perceived handling costs of larger numbers of
329 prey affected lizard response in our study. Nevertheless, in that scenario, we would still expect
330 animals to exhibit a preference, even if for the smaller amount of food. Additionally we would
331 have expected body size to influence lizards choices, as larger animals may be more adept at
332 handling a greater number of prey items. Miletto Petrazzini et al. (2017) did not report any effect
333 of body size, and age - a proxy for size - did not affect *L. guichenoti*'s *Choice* or *Interest* in our
334 study. The absence of preference in *P. siculus* and *L. guichenoti* suggests that the benefits of

selecting a larger number of prey are not different from selecting fewer. For instance, when considering hunting groups of 1 VS 4 or 2 VS 4 crickets, the probability of success might be similar. Furthermore, while one individual handles a prey item within the patch, other conspecifics could capture the rest. As a result, insectivorous lizards might not have evolved a preference for larger patches of prey, at least with small numbers (see Recio et al. 2021).

Conversely, our findings may be influenced by the experimental design. In our study, we rotated the position of the crickets in the choice with fewer items to create a situation where the numerical cues (fewer items) conflicted with other quantitative cues associated with numerosity. This approach was intended to control for other quantitative cues that could be used by lizards to make decisions (Cooper et al. 2024). However, this manipulation could have made the task more challenging for lizards, potentially affecting their performance. Indeed, it is possible that *L. guichenoti* uses multiple quantitative cues simultaneously during foraging decisions. When those cues conflict, the preference for one cue over another might vary between individuals, potentially producing the results observed here. Future studies should explore the use of other quantitative cues in *L. guichenoti* and how they interact with numerical information. Such studies would enhance our understanding of how *L. guichenoti* integrates different quantitative cues during foraging, providing insights into their decision-making processes and overall quantitative abilities.

Foraging behaviour is robust to early environmental experiences

Although the effects of early environment on cognition is widely studied, little attention has been paid to its impacts on numerical discrimination. To the best of our knowledge, there is only one study examining how developmental conditions influence numerical abilities (Vila Pouca et al.

2019). Given the potent effects of early environment on cognition and brain development (Szuran et al. 1994; Zhu et al. 2004; Amiel and Shine 2012; Clark et al. 2014; Amiel et al. 2014; Lui et al. 2017; Amiel et al. 2017), it is crucial to explore how early environmental conditions shape numerical abilities across taxa.

In our experimental design, prenatal treatments did not influence numerical discrimination in *L. guichenoti*, but we cannot discount the possibility that early environmental factors may affect numerical discrimination under other approaches or contexts. Based on previous studies showing the effects of prenatal temperature and GCs on cognitive abilities in reptiles and other groups (Szuran et al. 1994; Zhu et al. 2004; Amiel and Shine 2012; Clark et al. 2014; Amiel et al. 2014; Lui et al. 2017; Amiel et al. 2017), we predicted that lower incubation temperatures and exposure to CORT will impair lizards' performance, especially in those tests with higher ratios (Agrillo and Bisazza 2014; Nieder 2018). However, we did not find any effect of the experimental treatments on *L. guichenoti*'s behaviour when tested in a numerical task. Both GCs and thermal environment can exert differential effects on distinct areas of the brain. For example, in the eastern three-lined skink (*Bassiana dupeyerri*), elevated incubation temperatures increased cell density in the medial cortex - linked to learning process and memory - but did not impact cell density in other nuclei (Amiel et al. 2017). Similarly, impairments in spatial learning in rats (Haq et al. 2021) are likely associated with the greater density of GCs receptors in the hippocampus compared to other regions of the brain (Siegel 2006). Researchers have proposed that non-verbal number representation is processed by two types of systems: one for small sets (≤ 4) that tracks and encodes objects as individual entities, and another for larger numerosities that operate through estimation processes (Nieder 2005; Cantlon et al. 2009; Hyde 2011). While it remains unknown which areas of the reptiles' brain encode numerical information, our results suggest

that the brain regions involved in discrete numerical representation (≤ 4 items) might be robust to the effects of prenatal CORT and temperature in *L. guichenoti*.

Alternatively, it is plausible that treatment effects are masked by the overall absence of discrimination between numbers of prey items across most of the tests. In this regard, motivation is a key factor in spontaneous choice tests (Agrillo and Bisazza 2014; Bisazza et al. 2014). Both developmental CORT and incubation temperature can affect metabolic rate (hereafter MR) which could affect motivation in food based learning paradigms (Noble et al. 2018; Cossin-Sevrin et al. 2022; but see Crino et al. 2014). Although still possible, this explanation is less likely in our study because we controlled for motivation by providing only one cricket per day and fasting lizards for more than 20 hours before the test. In addition, we excluded tests where lizards did not interact with any of the options or did not eat the cricket provided after the test. Further studies should explore the effects of prenatal conditions on motivation and how it may affect the outcome of cognitive choice tests in reptiles.

Conclusion

Our study is, to the best of our knowledge, the first to explore the impact of prenatal conditions on numerical abilities in reptiles. Our findings suggest that *L. guichenoti* does not rely on numerical information for foraging decisions and that their performance does not vary due to prenatal treatments. These results indicate that early environmental conditions, such as incubation temperature and CORT exposure, may not affect numerosity in this species. However, it is critical to note that the methodologies employed in this study may not have fully captured the nuances of numerical cognition in *L. guichenoti*. Therefore, we recommend that future studies employ a broader array of tests and paradigms, including training-based tasks,

larger sets of stimuli, and other sensory modalities, to more comprehensively assess numerical competence in this and related species. Such work will be fundamental in unravelling early environmental factors' effects on reptiles' cognitive abilities and advancing our understanding of numerical discrimination across taxa.

Data accessibility

All data, data description, and R code are available in public repository https://github.com/Pablo-Recio/CORT-Temp_Numerical.

Declaration of AI use

We declare Chat GPT was used for questions related to coding and data analyses. All other parts of the manuscript were written by the authors and those parts where AI were used were checked and modified accordingly by the authors.

Authors' contributions

PR: conceptualization, methodology, data collection, data curation, formal analysis, writing—original draft, writing—review and editing; DL: data collection, writing—review and editing; OC: conceptualization, writing—review and editing; CF: conceptualization, funding acquisition, writing—review and editing; AP: data collection, writing—review and editing; DN: conceptualization, methodology, funding acquisition, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration

We declare we have no competing interests.

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Suplementary Material

Effects of treatments on numerical discrimination using the estimates and the raw data

Table S1.- Median and 95% Higest Density Intervals (95% HPDI) of the estimated probability of choosing first the higher amount (Choice) and the estimated interest for the higher amount of food (Interest) per treatment group for each of the numerical tests performed. 95% HPDI test the hypothesis that Choice = 0.5, and Interest = 0, which would indicate a preference towards one of choices.

		Choice		Interest	
Test	Treatment	Median	95% HPDI	Median	95% HPDI
1VS4	Cold-CORT (n = 20)	0.33	[0.11 , 0.57]	8.54	[-30.61 , 49.35]
		0.48	[0.23 , 0.72]	-2.77	[-40.84 , 34.65]
		0.60	[0.34 , 0.84]	-12.86	[-52.24 , 28.73]
		0.43	[0.2 , 0.69]	-4.39	[-42.39 , 35.1]
1VS3	Cold-CORT (n	0.39	[0.15 , 0.64]	-19.49	[-59.75 , 20.88]

		Choice		Interest	
Test	Treatment	Median	95% HPDI	Median	95% HPDI
	= 20)				
		0.65	[0.4 , 0.85]	-2.37	[-39.95 , 36.71]
		0.81	[0.61 , 0.96]	31.17	[-5.11 , 72.4]
		0.64	[0.38 , 0.87]	-8.24	[-49.13 , 31.43]
2VS4	Cold-CORT (n = 20)	0.50	[0.24 , 0.75]	12.55	[-27.29 , 52.49]
		0.48	[0.24 , 0.72]	-3.36	[-41.75 , 33.41]
		0.45	[0.21 , 0.71]	-9.97	[-48.1 , 29.59]
		0.55	[0.3 , 0.8]	8.66	[-30.96 , 47.03]
2VS3	Cold-CORT (n = 20)	0.60	[0.32 , 0.83]	-9.77	[-50.39 , 29.78]

		Choice		Interest	
Test	Treatment	Median	95% HPDI	Median	95% HPDI
		0.76	[0.53 , 0.93]	27.65	[-11.07 , 65.46]
		0.44	[0.19 , 0.7]	3.63	[-38.07 , 42]
		0.37	[0.13 , 0.61]	-29.13	[-69.44 , 7.53]
3VS4	Cold-CORT (n = 20)	0.41	[0.17 , 0.67]	-30.29	[-72.18 , 8.75]
		0.59	[0.34 , 0.82]	21.88	[-15.53 , 60.84]
		0.65	[0.39 , 0.87]	-14.01	[-54.51 , 27.79]
		0.70	[0.46 , 0.9]	14.21	[-26.74 , 52.95]

Table S2.- Performance of each treatment in each of the numerical tests using the raw data. For the variable Latency and Interest, we show the median and the 95% CI. For the varaible Choice, we show the proportion of individuals that chose the higher number of crickets first.

		Tests				
Variable	Treatment	1 VS 4	1 VS 3	2 VS 4	2 VS 3	3 VS 4
Latency	Hot-Control (n = 20)	595.5 [55.88 , 1593.72]	721.25 [192.5 , 2074.55]	622.8 [74.23 , 1447.22]	666.35 [84.63 , 2311.7]	742.2 [71.33 , 2344.85]
	Hot-CORT (n = 20)	1235.7 [257.98 , 4483.92]	1011.5 [203.12 , 2379.73]	852.4 [115.38 , 2527.57]	840.9 [98.2 , 2524.95]	1360.25 [145.43 , 6131.73]
	Cold-Control (n = 20)	663.35 [84.93 , 2605.7]	729.95 [92.8 , 3099.77]	630.5 [94.1 , 2500.82]	575 [70.75 , 1679.95]	491.15 [24.63 , 1915.57]
	Cold-CORT (n = 20)	880.25 [29.75 , 4557.85]	760.3 [60.08 , 3493.4]	703 [30.88 , 3522.77]	962.15 [35.93 , 4747.52]	1031.05 [19.88 , 4106.82]
Choice	Hot-Control (n = 20)	8	12	10	7	13
	Hot-CORT (n = 20)	11	15	9	9	11
	Cold-Control (n = 20)	10	13	10	15	12

		Tests				
Variable	Treatment	1 VS 4	1 VS 3	2 VS 4	2 VS 3	3 VS 4
	Cold-CORT (n = 20)	8	9	11	12	9
Interest	Hot-Control (n = 20)	-6.5 [-126.97 , 163.07]	-10.68 [-180.9 , 113.25]	6.6 [-85.05 , 162.3]	-31.1 [-118.62 , 56.62]	12.05 [-186.6 , 228.85]
	Hot-CORT (n = 20)	-15.11 [-152.65 , 61.45]	28.65 [-152.27 , 185.65]	-12.74 [-120.8 , 182]	1.68 [-108.4 , 120.55]	-15.71 [-148 , 84.4]
	Cold-Control (n = 20)	-2.75 [-141.42 , 204.65]	-3.05 [-152.55 , 179.07]	-3.95 [-142.12 , 104]	27.25 [-77.38 , 130]	21.55 [-101 , 164.22]
	Cold-CORT (n = 20)	12.9 [-188.15 , 195.67]	-14.15 [-203.8 , 123.02]	17.6 [-105.78 , 186.17]	-4.68 [-119.05 , 79.8]	-24.84 [-182.8 , 74.95]

Model results

Table S3A.- Summary of the model fitted for loglatency (Latency)

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_loglatency_Intercept	6.47	6.47	0.31	5.96	7.00	1.00	4547.93	6871.87
b_loglatency_test_type1VS3	-0.03	-0.03	0.21	-0.37	0.31	1.00	5851.20	8069.68
b_loglatency_test_type2VS4	-0.13	-0.13	0.21	-0.46	0.21	1.00	5607.53	8779.16
b_loglatency_test_type2VS3	-0.02	-0.02	0.21	-0.36	0.33	1.00	5792.88	8099.56
b_loglatency_test_type3VS4	-0.07	-0.07	0.21	-0.42	0.27	1.00	5394.76	8113.94
b_loglatency_tempHot	0.05	0.05	0.45	-0.69	0.80	1.00	4240.55	6468.22
b_loglatency_cortCo	0.09	0.09	0.35	-0.50	0.66	1.00	3501.90	6160.76

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
ntrol								
b_loglatency_sexm	-0.46	-0.45	0.22	-0.82	-0.10	1.00	5456.02	7259.40
b_loglatency_age	0.06	0.06	0.02	0.02	0.10	1.00	5242.87	6844.75
b_loglatency_test_type1VS3:tempHot	-0.03	-0.03	0.30	-0.52	0.46	1.00	5246.33	7514.61
b_loglatency_test_type2VS4:tempHot	-0.18	-0.18	0.30	-0.67	0.31	1.00	5432.60	8115.85
b_loglatency_test_type2VS3:tempHot	-0.25	-0.25	0.30	-0.74	0.25	1.00	5517.51	7473.31
b_loglatency_test_type3VS4:tempHot	-0.10	-0.11	0.30	-0.60	0.39	1.00	5247.17	7570.16
b_loglatency_test_ty	0.04	0.04	0.29	-0.43	0.53	1.00	5376.57	7715.18

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
pe1VS3:cortControl								
b_logla tency_test_type2VS4:cortControl	0.15	0.16	0.29	-0.33	0.64	1.00	5492.31	8062.19
b_logla tency_test_type2VS3:cortControl	-0.04	-0.04	0.29	-0.52	0.44	1.00	5459.05	7755.73
b_logla tency_test_type3VS4:cortControl	-0.38	-0.38	0.29	-0.87	0.09	1.00	5264.74	8063.37
b_logla tency_tempHot:cortControl	-0.78	-0.78	0.49	-1.58	0.03	1.00	3491.24	5908.08
b_logla tency_test_type1VS3:tempHot:cortControl	0.25	0.25	0.42	-0.43	0.94	1.00	5103.28	7366.37

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_loglatency_test_type2VS4:tempHot:corttControl	0.28	0.28	0.42	-0.40	0.97	1.00	4860.78	7733.09
b_loglatency_test_type2VS3:tempHot:corttControl	0.37	0.37	0.42	-0.32	1.05	1.00	5243.01	8532.21
b_loglatency_test_type3VS4:tempHot:corttControl	0.74	0.75	0.42	0.05	1.44	1.00	5119.54	7726.59

586

587 *Table S3B.- Summary of the model fitted for Choice*

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_choice_Intercept	-0.68	-0.68	0.58	-1.64	0.26	1.00	4717.07	6912.29
b_choice_test_type1	0.24	0.24	0.69	-0.89	1.38	1.00	5106.43	7093.98

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
VS3								
b_choice_test_type2 VS4	0.71	0.70	0.69	-0.42	1.86	1.00	4984.93	7514.81
b_choice_test_type2 VS3	1.12	1.10	0.73	-0.06	2.35	1.00	4902.86	6926.45
b_choice_test_type3 VS4	0.33	0.32	0.70	-0.82	1.48	1.00	5219.20	7158.11
b_choice_tempHot	1.13	1.12	0.83	-0.23	2.50	1.00	4283.18	6599.41
b_choice_cortControl	0.61	0.60	0.74	-0.59	1.84	1.00	3922.51	6114.01
b_choice_sexm	-0.06	-0.06	0.30	-0.54	0.43	1.00	14505.46	8697.98
b_choice_age	-0.04	-0.04	0.03	-0.09	0.01	1.00	12392.71	8820.47
b_choice_test_type1 VS3:temp	0.80	0.79	1.04	-0.88	2.55	1.00	4972.18	6745.87

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_choice_test_type2VS4:tempHot	-1.33	-1.33	1.00	-2.98	0.29	1.00	4886.89	7191.48
b_choice_test_type2VS3:tempHot	-1.77	-1.77	1.04	-3.49	-0.09	1.00	4289.06	6024.53
b_choice_test_type3VS4:tempHot	-0.13	-0.13	1.02	-1.80	1.57	1.00	4538.82	7185.57
b_choice_test_type1VS3:cortControl	0.48	0.47	0.99	-1.15	2.12	1.00	4938.94	7818.41
b_choice_test_type2VS4:cortControl	-0.70	-0.69	0.98	-2.32	0.90	1.00	4749.40	7672.85
b_choice_test_type2VS3:cortC	0.15	0.16	1.04	-1.53	1.85	1.00	4980.80	8127.50

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
ontrol								
b_choice_test_type3 VS4:cortControl	0.14	0.13	1.00	-1.50	1.77	1.00	4881.82	7388.95
b_choice_tempHot:cortControl	-1.33	-1.32	1.06	-3.06	0.40	1.00	3598.45	6418.88
b_choice_test_type1 VS3:tempHot:cortControl	-0.65	-0.64	1.44	-3.01	1.71	1.00	4947.05	7258.07
b_choice_test_type2 VS4:tempHot:cortControl	1.82	1.79	1.41	-0.52	4.12	1.00	4832.87	7213.76
b_choice_test_type2 VS3:tempHot:cortControl	0.25	0.25	1.45	-2.12	2.59	1.00	4689.39	7011.19

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_choice_test_type3VS4:tempHot:cortControl	0.81	0.80	1.44	-1.57	3.20	1.00	4767.29	7038.46

588

589 *Table S3C.- Summary of the model fitted for Interest*

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_comparedinterest_Intercept	12.58	12.75	20.03	-20.62	45.52	1.00	4226.87	6914.84
b_comparedinterest_test_type1VS3	-27.45	-27.46	24.85	-68.46	13.12	1.00	5057.66	7251.20
b_comparedinterest_test_type2VS4	4.45	4.37	25.04	-36.11	45.40	1.00	4955.22	7144.03
b_comparedinterest_test_type2	-18.02	-18.08	25.03	-59.03	23.55	1.00	4608.81	7655.14

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
VS3								
b_compar edinterest_ test_type3	-38.45	-38.44	25.60	-80.23	3.89	1.00	4974.12	7540.36
VS4								
b_compar edinterest_ tempHot	-21.42	-21.25	28.74	-68.59	25.65	1.00	3724.65	6038.13
b_compar edinterest_ cortContro l	-10.87	-10.78	25.67	-53.25	31.15	1.00	3414.61	5708.73
b_compar edinterest_ sexm	-8.60	-8.64	9.32	-23.92	7.03	1.00	17607.01	8664.01
b_compar edinterest_ age	-0.68	-0.67	0.97	-2.25	0.91	1.00	15664.51	9250.40
b_compar edinterest_ test_type1	71.62	71.63	35.19	13.31	129.60	1.00	4600.90	6586.40
VS3:temp Hot								

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
b_comparedinterest_test_type2VS4:tempHot	-1.40	-1.38	35.97	-59.74	58.37	1.00	4594.12	6529.68
b_comparedinterest_test_type2VS3:tempHot	34.51	34.43	35.89	-23.35	93.63	1.00	4206.05	6855.33
b_comparedinterest_test_type3VS4:tempHot	37.54	37.66	36.94	-22.35	97.98	1.00	4702.14	7554.35
b_comparedinterest_test_type1VS3:cortControl	27.46	27.64	35.35	-30.54	86.37	1.00	4866.97	7599.14
b_comparedinterest_test_type2VS4:cortC	-5.20	-5.27	35.16	-62.72	52.41	1.00	4362.77	6797.39

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
ontrol								
b_compar edinterest_ test_type2 VS3:cortC ontrol	48.43	48.30	35.49	-9.54	106.81	1.00	4047.11	6637.73
b_compar edinterest_ test_type3 VS4:cortC ontrol	63.11	62.88	35.72	4.22	122.72	1.00	4618.01	7533.53
b_compar edinterest_ tempHot:c ortControl	19.41	19.72	36.63	-40.29	79.85	1.00	3346.75	5960.45
b_compar edinterest_ test_type1 VS3:temp Hot:cortC ontrol	-75.56	-75.33	50.52	-157.46	7.56	1.00	4608.18	7070.71
b_compar edinterest_ test_type2	15.19	15.16	50.30	-69.06	97.00	1.00	4217.24	6394.78

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
VS4:tempHot:cortControl								
b_comparedinterest_test_type2	-89.54	-88.78	50.35	-173.98	-6.66	1.00	3896.04	6636.15
VS3:tempHot:cortControl								
b_comparedinterest_test_type3	-43.38	-43.71	51.44	-127.24	41.39	1.00	4491.70	6562.71
VS4:tempHot:cortControl								

590

591 *Table S3D.- Summary of the model fitted for other effects*

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
sd_clutch__loglatency_Intercept	0.34	0.34	0.19	0.04	0.67	1.01	716.16	2313.13

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
sd_lizard_id__loglatency_Intercept	0.81	0.81	0.10	0.64	0.97	1.00	1725.51	2777.99
sd_clutch__choice_Intercept	0.26	0.23	0.18	0.02	0.60	1.00	3913.70	5803.67
sd_lizard_id__choice_Intercept	0.59	0.60	0.25	0.13	0.99	1.00	2047.51	3110.77
sd_clutch__comparedinterest_Intercept	9.92	9.22	6.32	1.03	21.27	1.00	4065.36	6333.93
sd_lizard_id__comparedinterest_Intercept	9.31	8.34	6.38	0.81	20.85	1.00	3847.09	5748.30
sigma_loglatency	0.65	0.65	0.03	0.61	0.70	1.00	12611.43	8861.89
sigma_comparedinterest	78.78	78.70	3.03	73.99	83.90	1.00	15359.76	8383.11

variable	mean	median	sd	q5	q95	rhat	ess_bulk	ess_tail
lprior	-29.26	-29.25	0.08	-29.40	-29.13	1.00	8343.29	9738.65
lp__	-3440.63	-3440.02	18.68	-3472.06	-3410.58	1.00	2890.39	5974.94

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Testing potential side biases

_Table S4.- Number of individuals per treatment that chose the right (R) or left (L) side in each of the numerical tests. p-value indicates the result of the binomial test comparing the number of choices between sides.

	1 VS 4	1 VS 3	2 VS 4	2 VS 3	3 VS 4
Control-Cold (n = 20)	R = 12 | L = 8 , p = 0.5	R = 12 | L = 8 , p = 0.5	R = 9 | L = 11 , p = 0.82	R = 12 | L = 8 , p = 0.5	R = 12 | L = 8 , p = 0.5
Control-Hot (n = 20)	R = 12 | L = 8 , p = 0.5	R = 5 | L = 15 , p = 0.04	R = 9 | L = 11 , p = 0.82	R = 10 | L = 10 , p = 1	R = 11 | L = 9 , p = 0.82
CORT-Cold (n = 20)	R = 11 | L = 9 , p = 0.82	R = 11 | L = 9 , p = 0.82	R = 11 | L = 9 , p = 0.82	R = 10 | L = 10 , p = 1	R = 11 | L = 9 , p = 0.82
CORT-Hot (n = 20)	R = 11 | L = 9 , p = 0.82	R = 9 | L = 11 , p = 0.82	R = 9 | L = 11 , p = 0.82	R = 10 | L = 10 , p = 1	R = 11 | L = 9 , p = 0.82

Prey orientation test

We performed a choice test to investigate if the orientation of crickets affected the lizards' choice. We used the same platform and Petri dish as in the numerical discrimination task, but we placed one cricket on each side of the platform, one oriented vertically in respect to the shelter position (see Fig. 1 B) and the other one horizontal to the shelter. We performed a unique trial randomising the position of the orientation of the cricket. We recorded the number of individuals that chose the cricket oriented vertically and horizontally. Results are shown in the table below.

Table S4.- Number of individuals per treatment that chose the cricket oriented horizontally (Horizontal) or vertically (Vertical) in our control tests. p-value indicates the result of the binomial test comparing the number of choices between both choices.

	Horizontal	Vertical	p-value
Cold-Control (n = 20)	8	12	0.503
Hot-Control (n = 20)	7	13	0.263
Cold-CORT (n = 20)	12	8	0.503
Hot-CORT (n = 20)	9	11	0.824

Searching for relevant literature

To look for relevant literature about the effects of early environment on numerical discrimination, we searched in the Web of Science and Scopus using the terms on respectively. The queries employed are detailed below. We looked for manuscripts for all years available. The last search was conducted on the 11th of October 2024; the results below refer to this last search.

We found 24 articles on Web of Science and 22 on Scopus, plus one preprint on Scopus. Out of all the documents, only one study was relevant to our search: Vila Pouca et al. (2019). That study appeared in both databases and was the only one that explored the effects of early environment on numerical abilities.

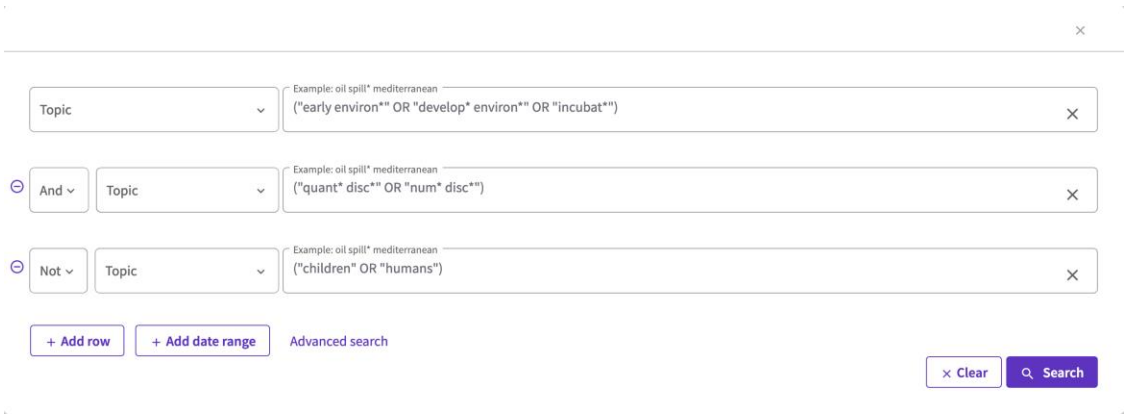


Fig 3— Search query on Web of Science.

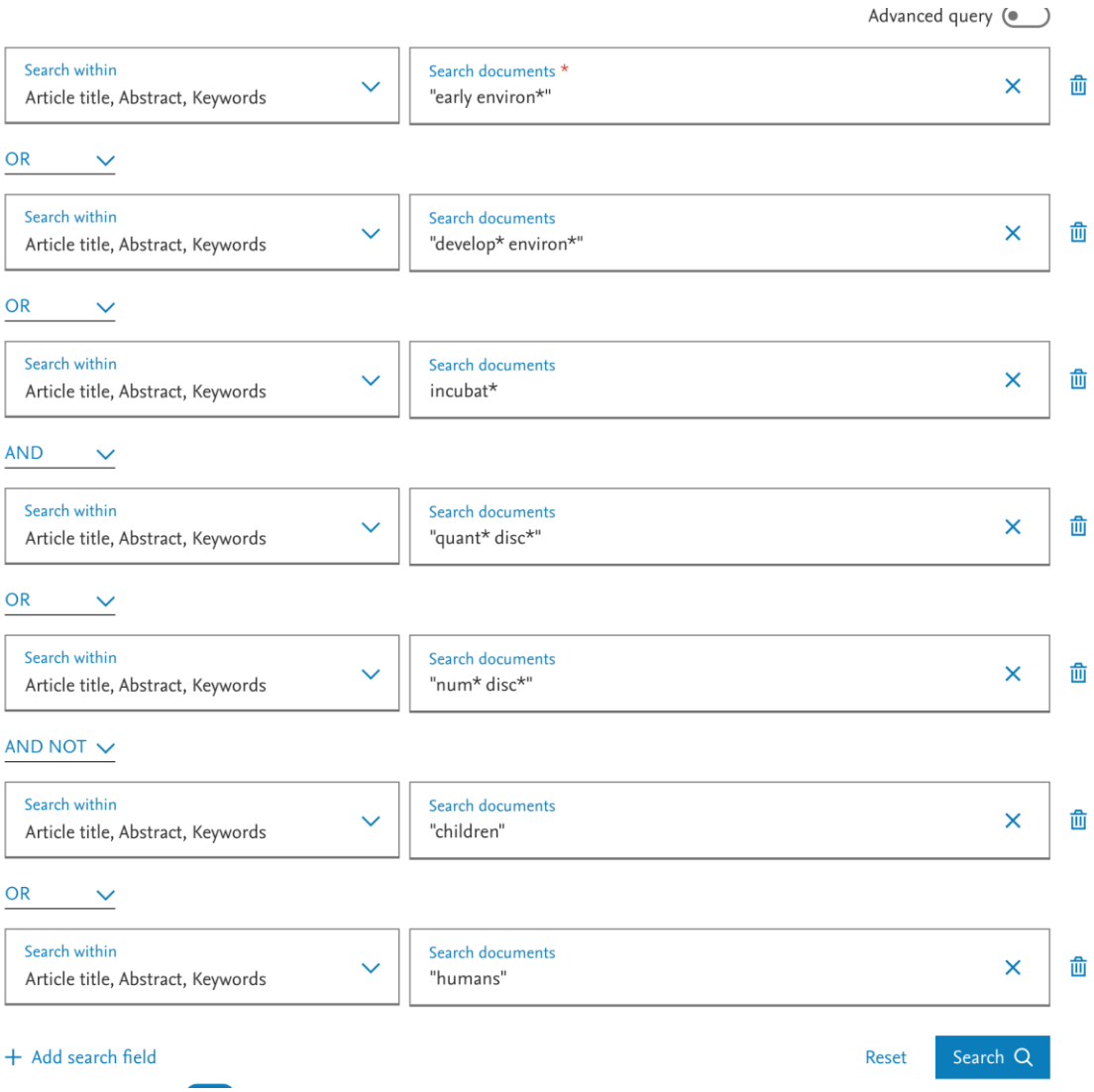
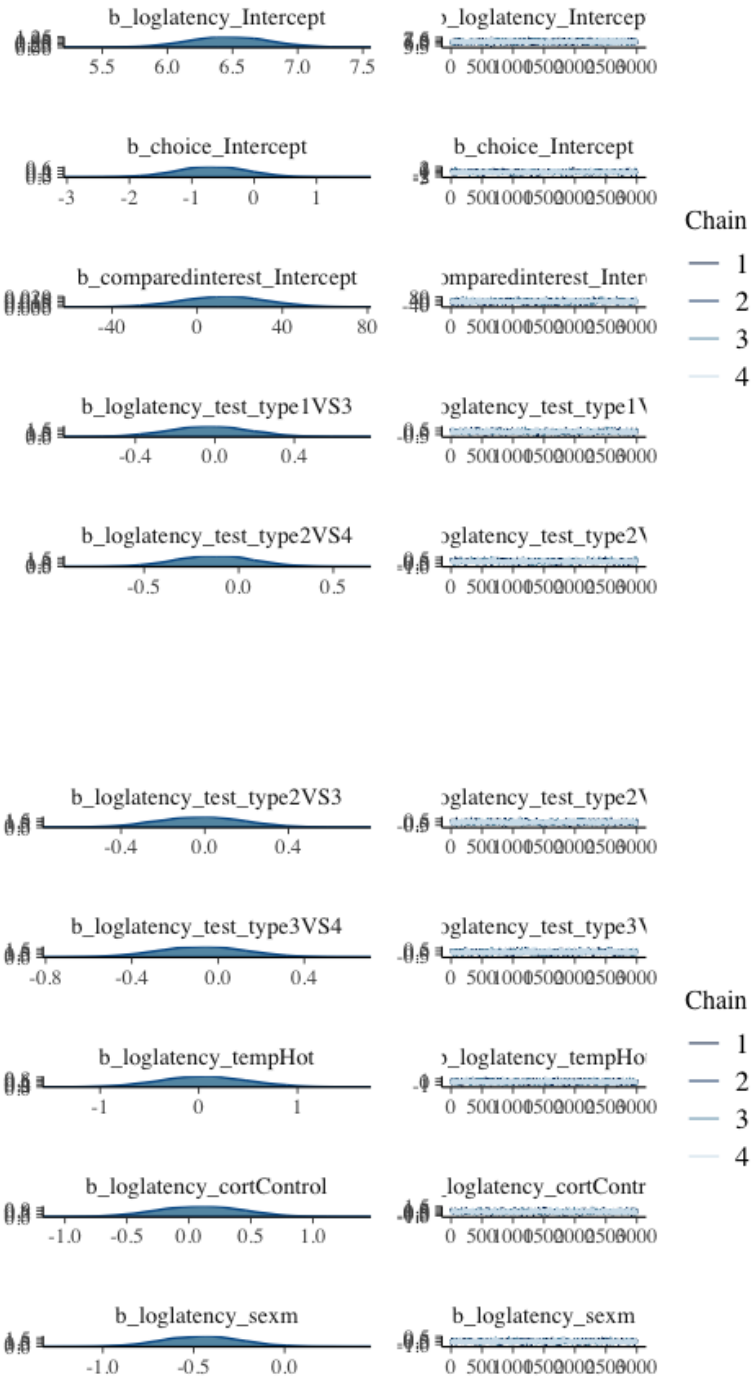


Fig 4— Search query on Scopus.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b_loglatency_test_type3VS4
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b_loglatency_cortControl
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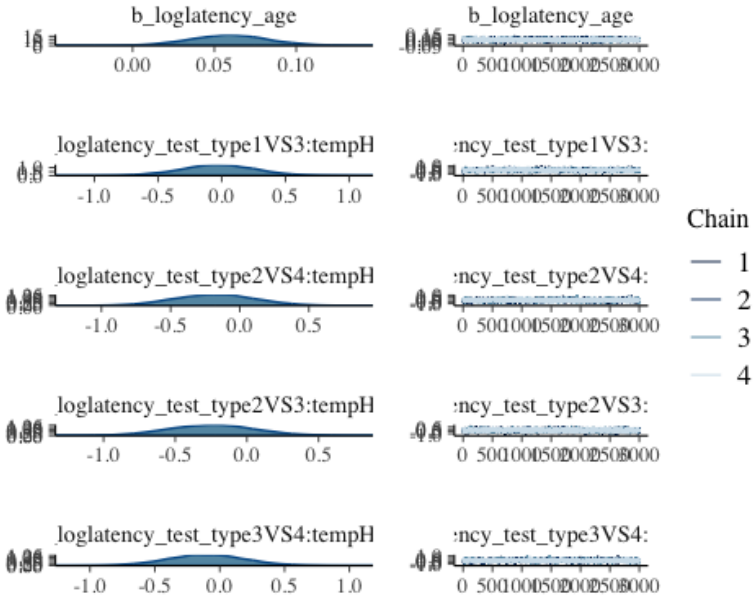
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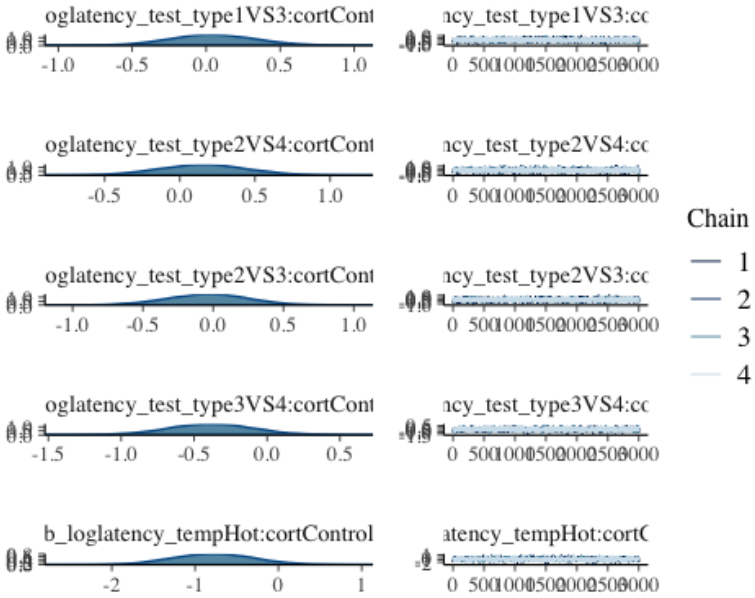
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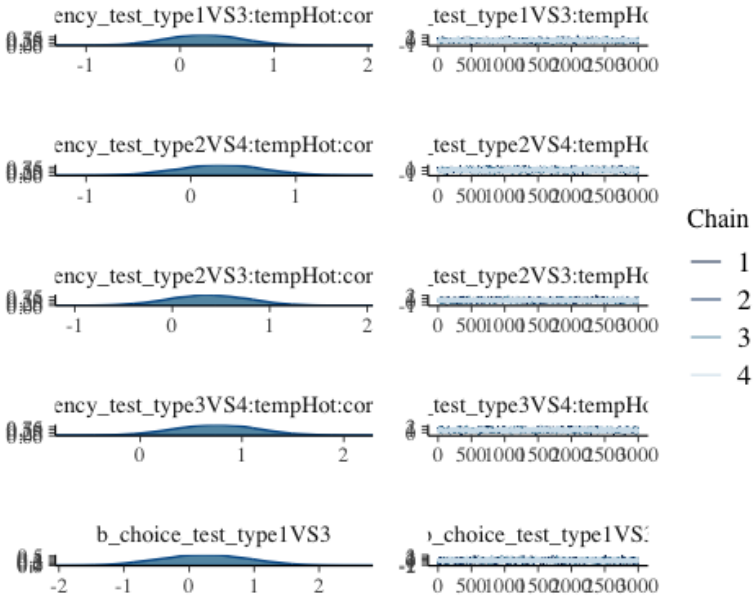
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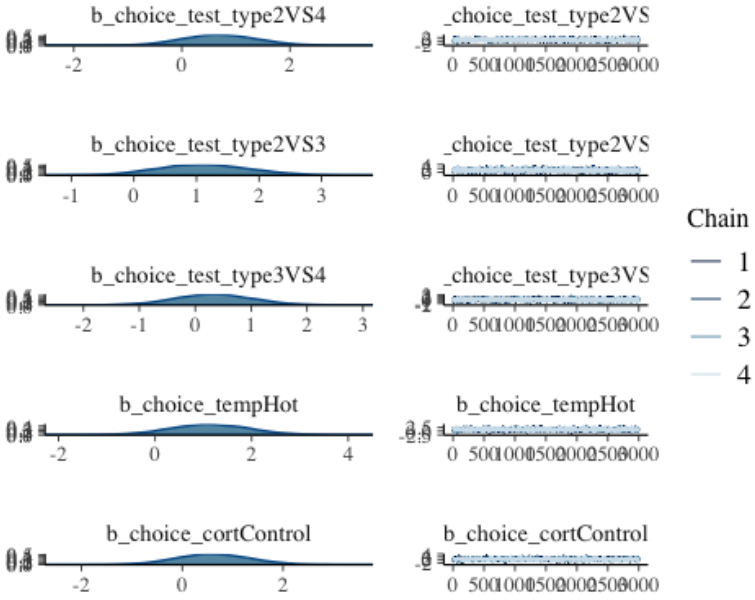
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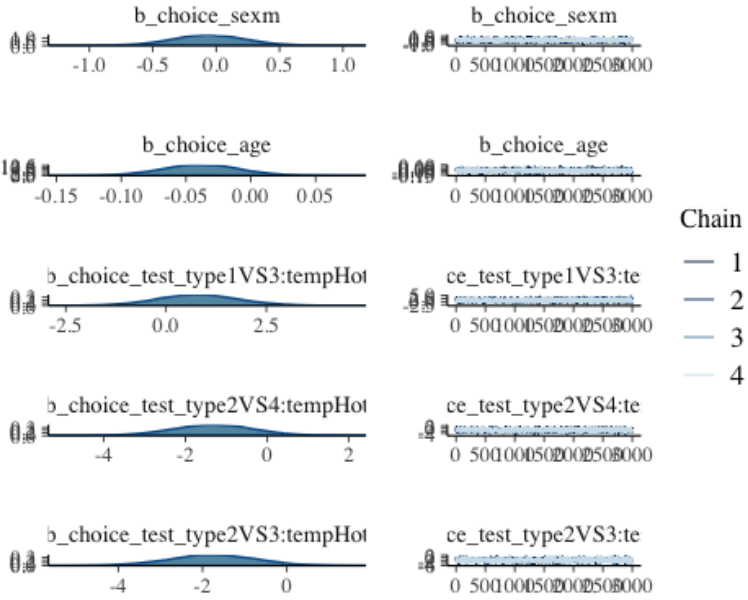
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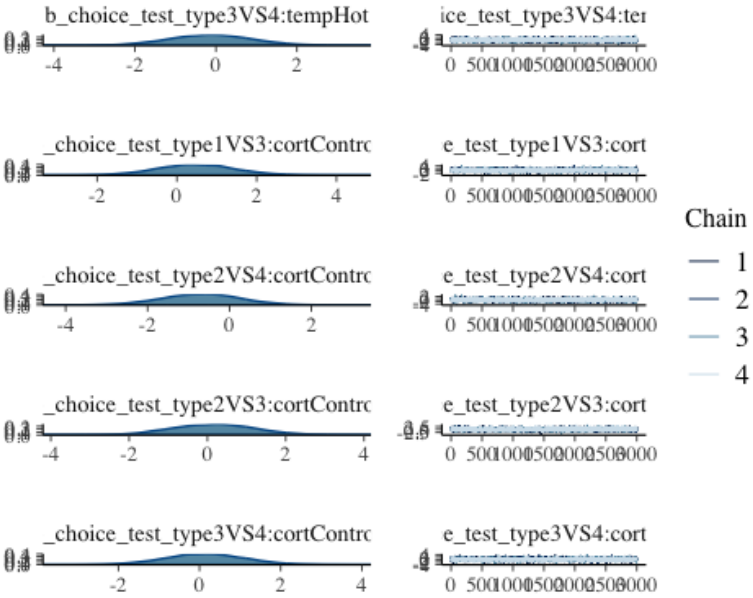
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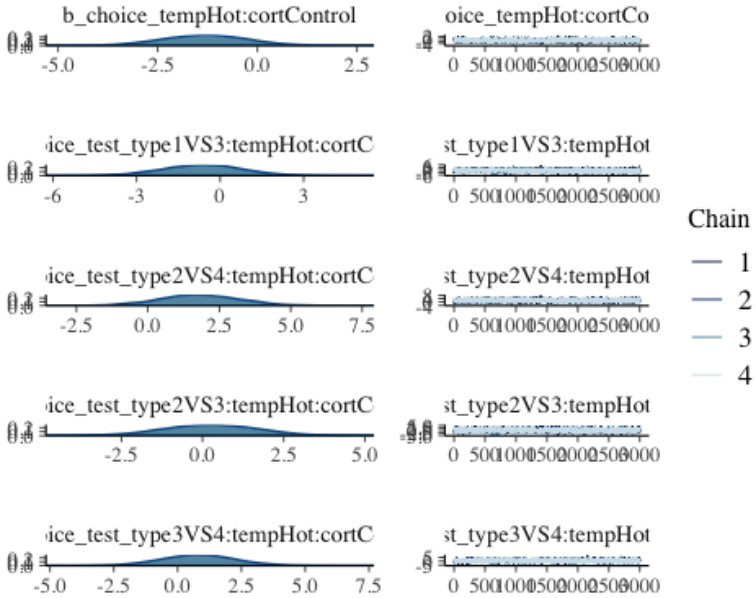
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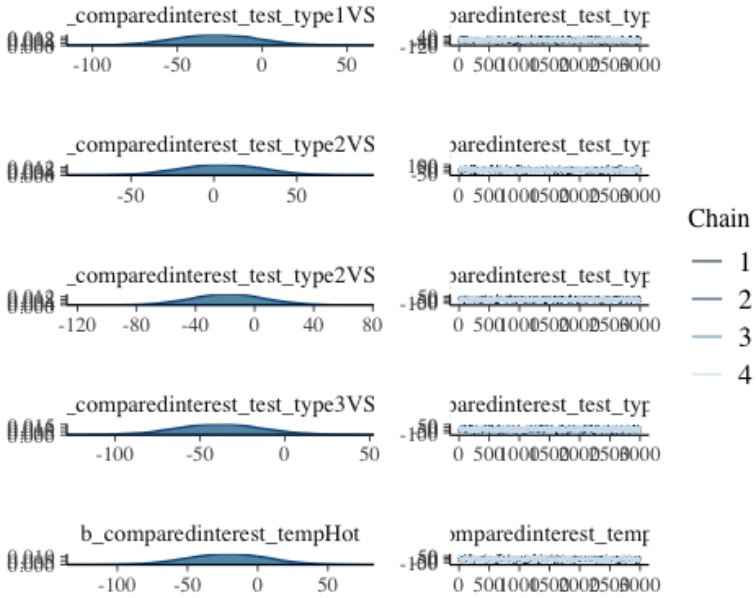
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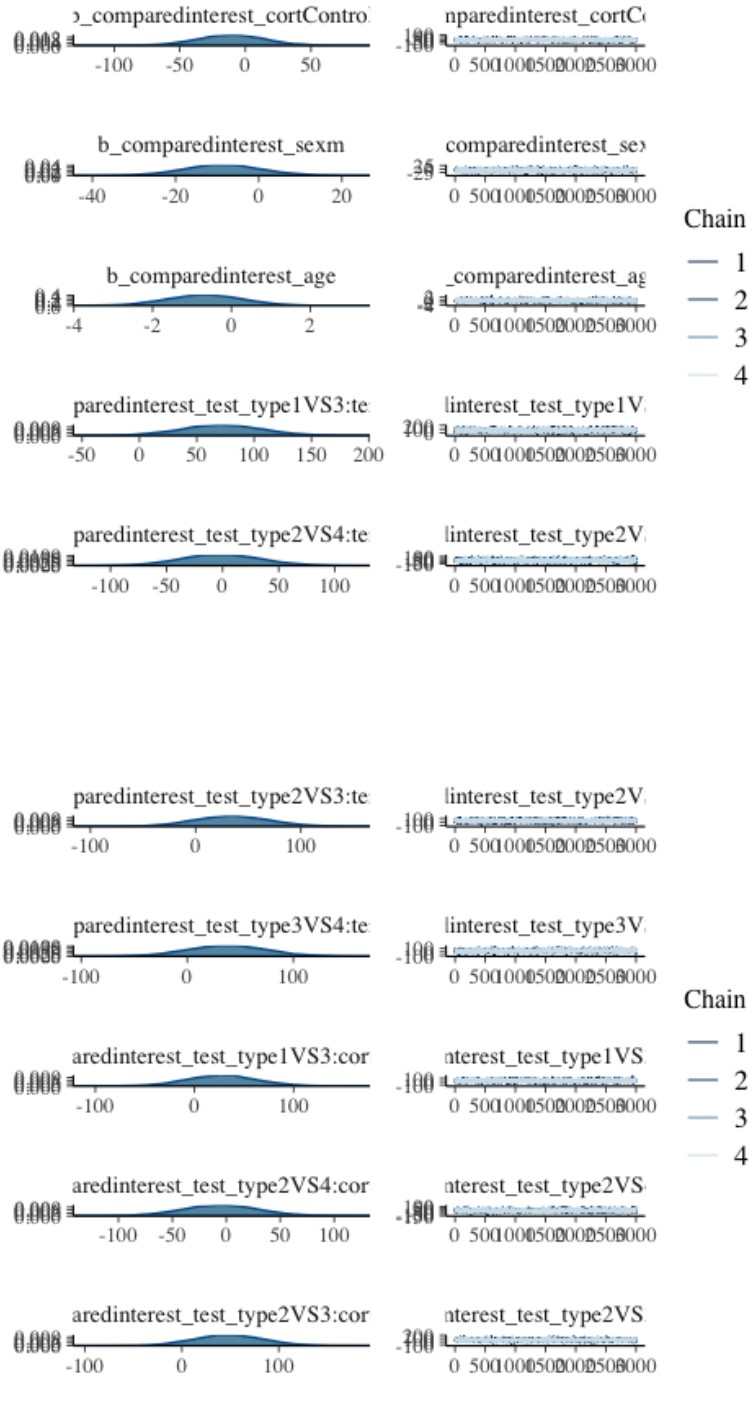
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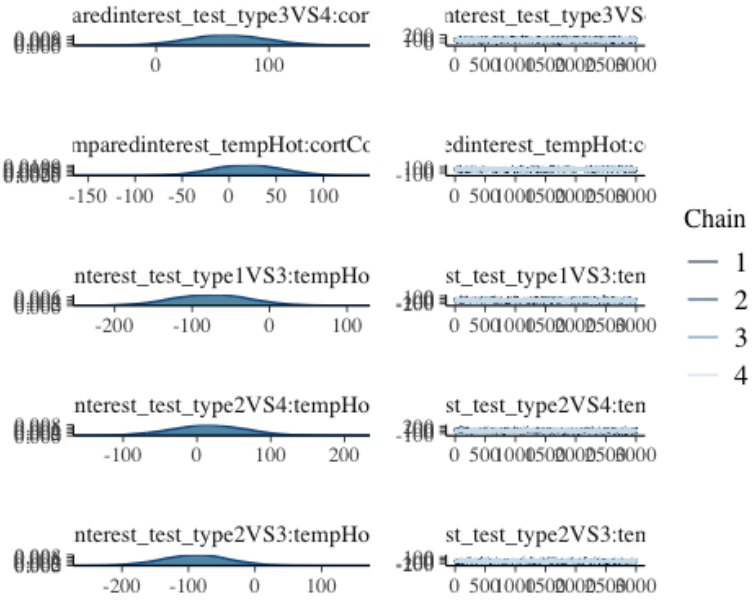
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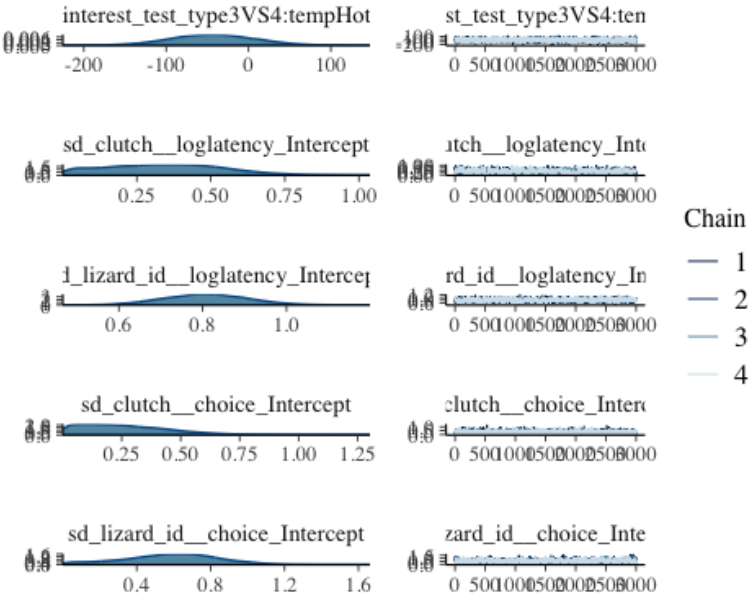
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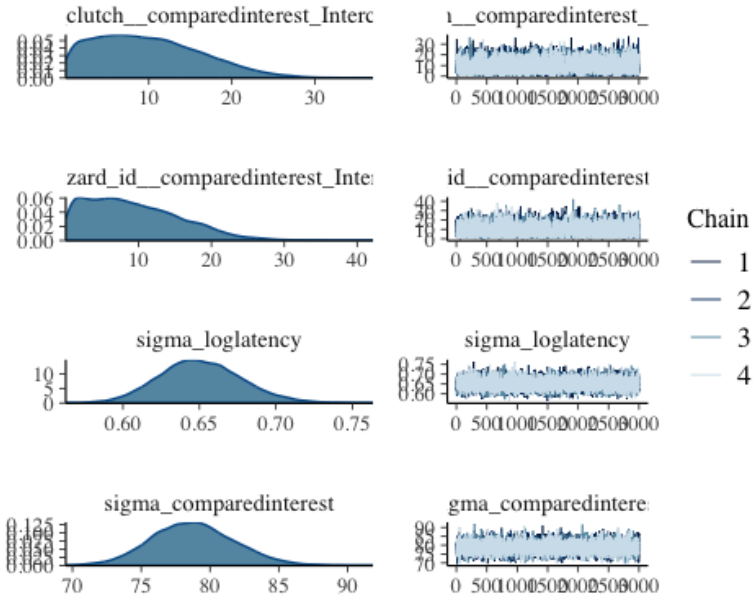
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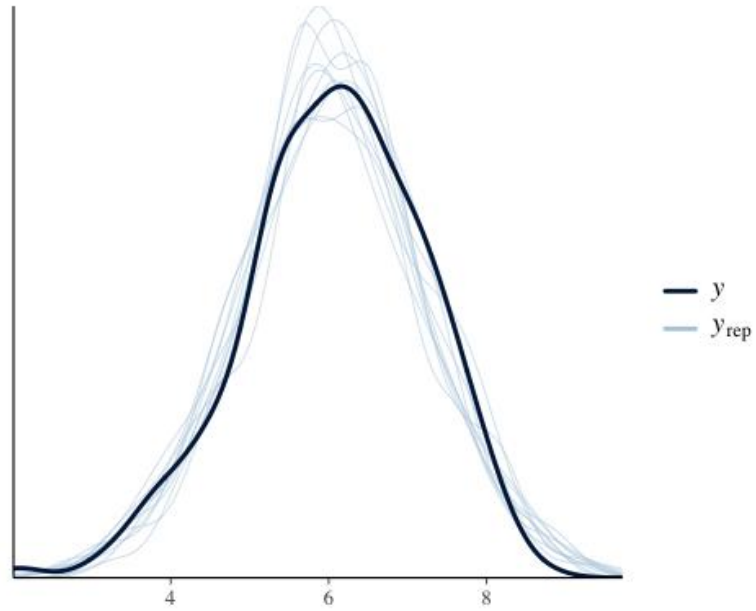
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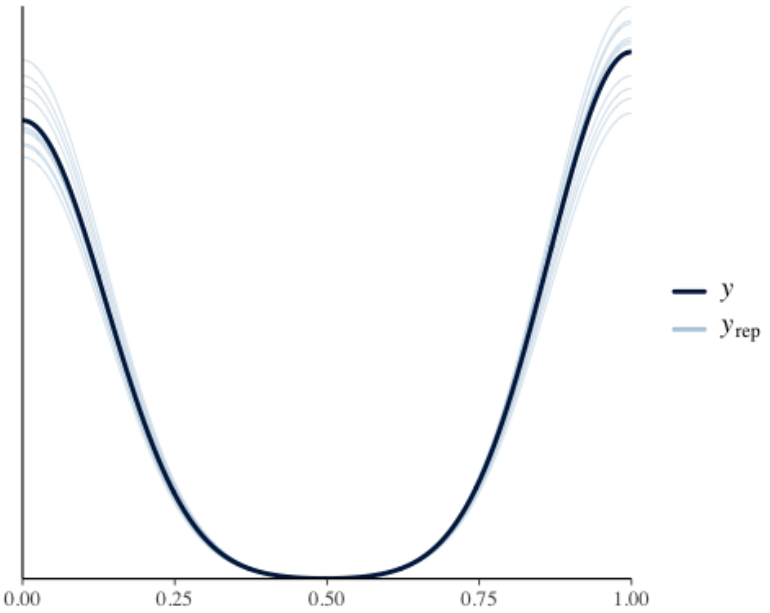

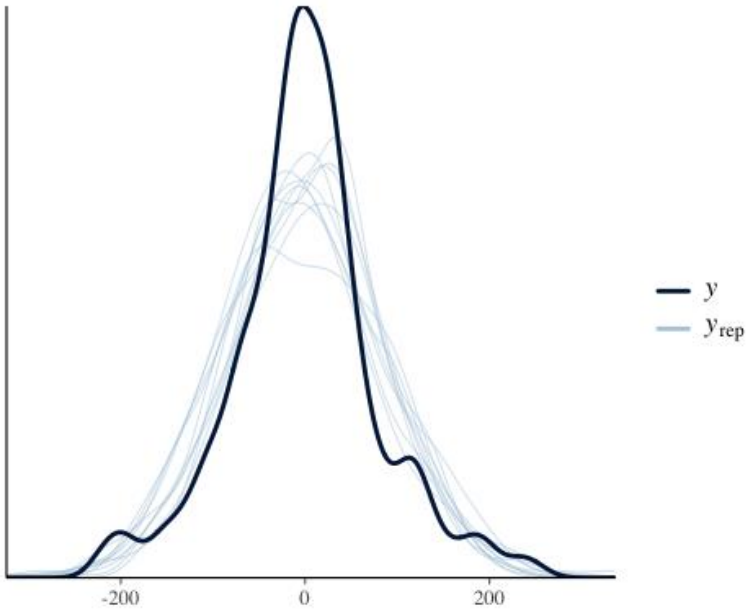

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Figure

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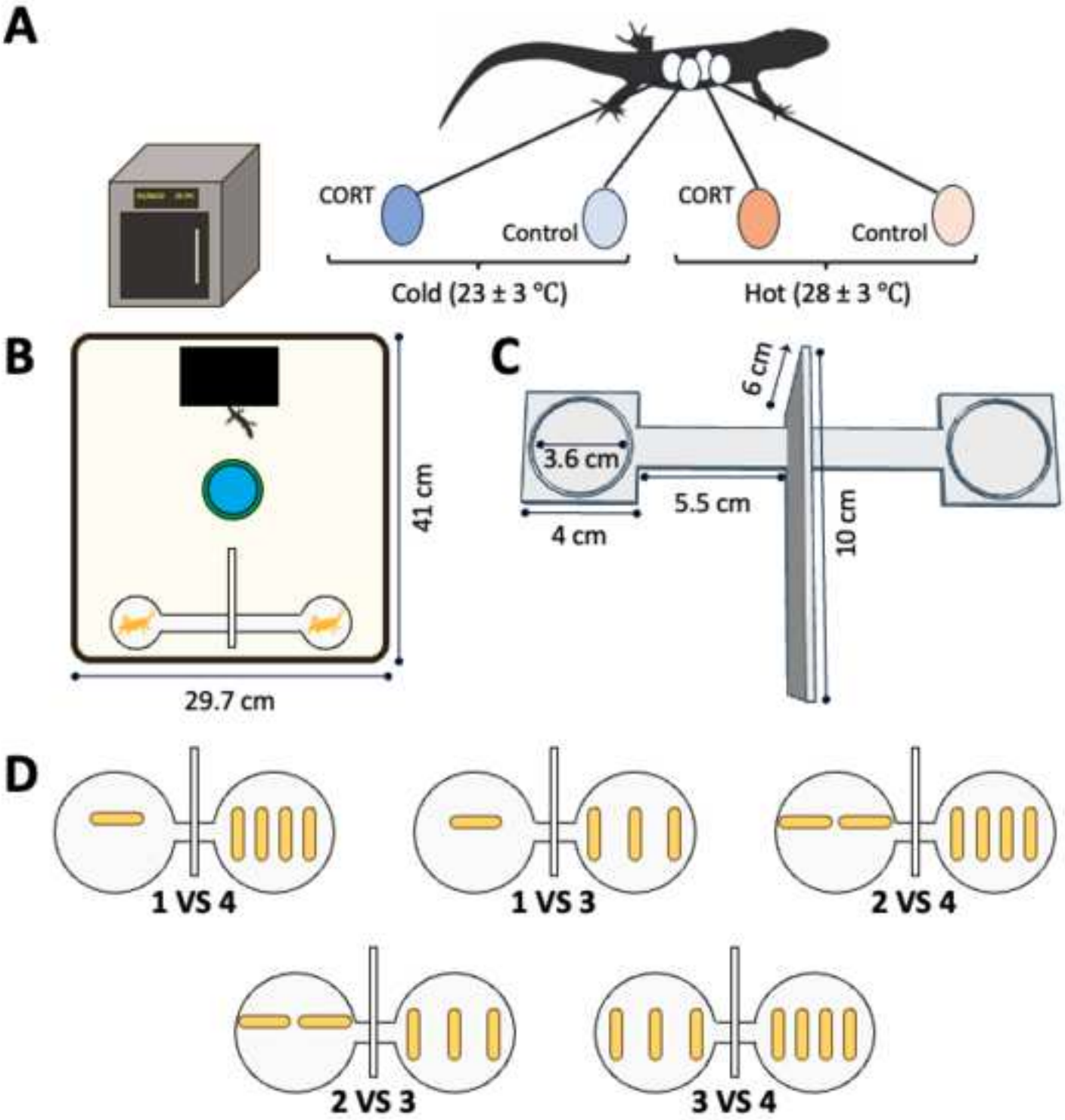

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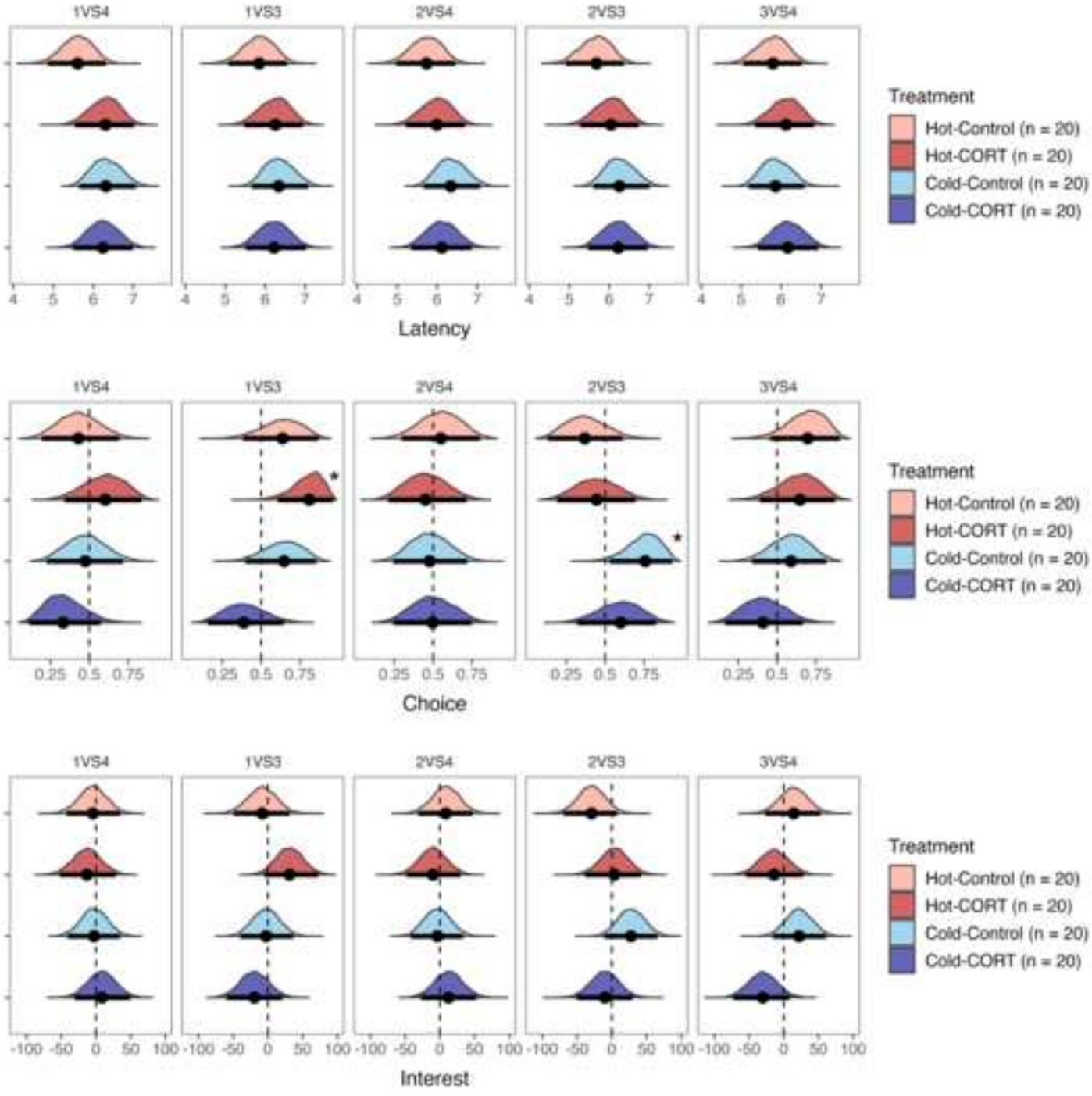
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